

THE EFFECTIVENESS OF THE ALERE ISCREEN© URINE ADULTERATION TEST STRIP AT
DETECTING SIX COMMON URINE ADULTERANTS

by

Carrie Beard

A Senior Honors Project Presented to the

Honors College

East Carolina University

In Partial Fulfillment of the

Requirements for

Graduation with Honors

by

Carrie Beard

Greenville, NC

May 2016

Approved by:

Ann McConnell, MAEd, MLS(ASCP)^{CM}

Department of Clinical Laboratory Science

College of Allied Health Sciences

The Effectiveness of the Alere iScreen© Urine Adulteration Test Strip at Detecting Six Common
Urine Adulterants

Author: Carrie Beard

Department of Clinical Laboratory Science, East Carolina University, Greenville, NC, USA

Faculty Advisor: Ann McConnell, MAEd, MLS(ASCP)^{CM}

Abstract

In 1988, the United States government published the first federal regulations for the testing of human samples for the presence of illegal drugs.¹ These regulations produced successful techniques for detecting illegal substances in human samples, specifically urine. However, they also caused an increase in methods used to falsify the results of these tests. The most common of these methods is that of *in vitro* adulteration, or adding a substance to a urine sample in order to cause a false negative test result. As a result of these adulteration methods, new tests have been developed to assess the integrity of samples collected for drug of abuse testing. To examine the effectiveness of one specimen validity test, the Alere iScreen© Urine Adulteration Test Strip, different concentrations of six common adulterants were created and combined with drug-positive human urine controls to simulate adulterated samples. Solid meat tenderizer, liquid meat tenderizer, lemon juice, apple cider vinegar, eye drops, and bleach were added at varying dilutions to Alere's positive iScreen© Urine Control. Each sample was tested for pH, specific gravity, nitrite, glutaraldehyde, creatinine, and pyridinium chlorochromate/oxidant presence by the test strip in duplicate to determine if the specimen validity test was effective at detecting the presence of the adulterants. The experimentation demonstrated that the iScreen© Urine Adulteration Test Strips were capable of detecting bleach, eye drops, lemon juice, and apple cider vinegar in urine at differing dilutions. It also

demonstrated that liquid and solid meat tenderizers could not be detected when added to the controls.

Introduction

The implementation of federal guidelines for pre-employment and random drug of abuse screening tests on human samples, such as urine, hair, or saliva, had a radical effect on the workplace as a whole. These guidelines were implemented under President Ronald Reagan in 1988, with the goals of discouraging potential and current employees from using illicit drugs and promoting a safe and responsible work environment. The methods of identifying drugs of abuse, specifically in urine samples, include immunoassays and gas chromatography/mass spectrometry (GC/MS).¹ These methods have proved effective at detecting the presence of commonly used illegal substances. However, with the implementation of these effective methods came a surge in attempts to find ways to falsify the results of these drugs screens.

One of the most common methods of falsifying drug screen results is the use of adulterants, or substances either ingested by the individual (*in vivo*) or added to the urine (*in vitro*) that interfere with the screening methods and in turn mask the presence of drugs of abuse.² The most common adulterants utilized are *in vitro* methods that can be readily found by an individual attempting to falsify drug screen results. These are usually common household products, such as bleach or liquid hand soap, but there are several commercial products specifically designed to produce false negative results as well.³ The use of adulterants poses a serious problem to the validity of screening tests and has resulted in the usage of physical and chemical tests to detect the presence of these contaminants. One such testing method is the adulteration test strip, a urine dipstick containing multiple reagent test pads that determine the validity of a urine sample through chemical reactions that produce a visible result on the strip.⁴

Several studies have been performed assessing the accuracy of certain test strips, such as the Intect® 7 or AdultaCheck 4, at detecting a variety of household and commercial adulterants.^{4,5} While these specimen validity tests were found to be effective at detecting the presence of certain common adulterants, there are new adulteration strips and adulteration methods available that have yet to be discussed in the existing specimen validity literature.

The objective of this research was to utilize an existing specimen validity testing method, the Alere iScreen© Urine Adulteration Test Strip, and test its effectiveness at detecting current *in vitro* adulteration methods used to mask drug-positive urine samples. It was hypothesized that the urine adulteration test strips would be effective at detecting the presence of the adulterants in the samples. The adulterants used were six common household items: powdered meat tenderizer, liquid meat tenderizer, lemon juice, apple cider vinegar, Visine eye drops, and bleach that were added in varied concentrations to a drug-positive, stabilized human urine control to simulate an adulterated urine sample. This research will be important in evaluating the adulteration strip's accuracy and the ability of the tested adulterants to evade detection.

Materials and Methods

Alere iScreen© Urine Adulteration Test Strips (#MLA5070003, Alere, Portsmouth, VA, USA) were the specimen validity test utilized in this research. The adulteration test strips evaluated the urine based on six characteristics: pH, specific gravity, nitrite, glutaraldehyde, creatinine, and pyridinium chlorochromate/oxidant presence. Alere iScreen© Urine Controls (#KN183, Alere, San Diego, CA, USA) were utilized in the experimentation and they consisted of stabilized human urine. The positive control contained added reference drug metabolites that were verified by the manufacturer to have a minimum of 98% purity. The positive control contained the following target concentrations of drug metabolites: Amphetamine (AMP)

3000ng/mL; Methamphetamine (MET) 3000ng/mL; MDMA 1500ng/mL; Barbiturates (BAR) 900ng/mL; Benzodiazepines (BZO) 900ng/mL; Buprenorphine (BUP) 30ng/mL; Cocaine (COC) 900ng/mL; Methadone (MTD) 900ng/mL; Methaqualone 900ng/mL; PCP 75ng/mL; Opiates (MOP) 6000ng/mL; Oxycodone (OXY) 300ng/mL; Propoxyphene 900ng/mL; THC 150ng/mL; Tricyclic Antidepressants (TCD) 3000ng/mL. The negative control was verified by the manufacturer to contain 0ng/mL of the drugs present in the positive control. The positive and negative controls were used to ensure the accuracy of the test strip and to create the simulated urine samples and were tested before testing the adulterants began.

The adulterants used were Tradewinds Meat Tenderizer (Amerifoods Trading Co.), Liquid Meat Tenderizer (3V Company), Visine eye drops (Johnson & Johnson), Clorox bleach (S.C. Johnson Inc.), White House Apple Cider Vinegar (National Fruit Product Co.), and lemon juice extracted from a whole lemon. Other necessary items included 0.85% Isotonic Buffered Blood Bank Saline (#584131, Fisher Diagnostics, Middletown, VA) for dilution, micropipettes, pipet tips, 12x75 mm test tubes, the Sartorius MC1 scale, weigh boats, and sterile urine collection cups.

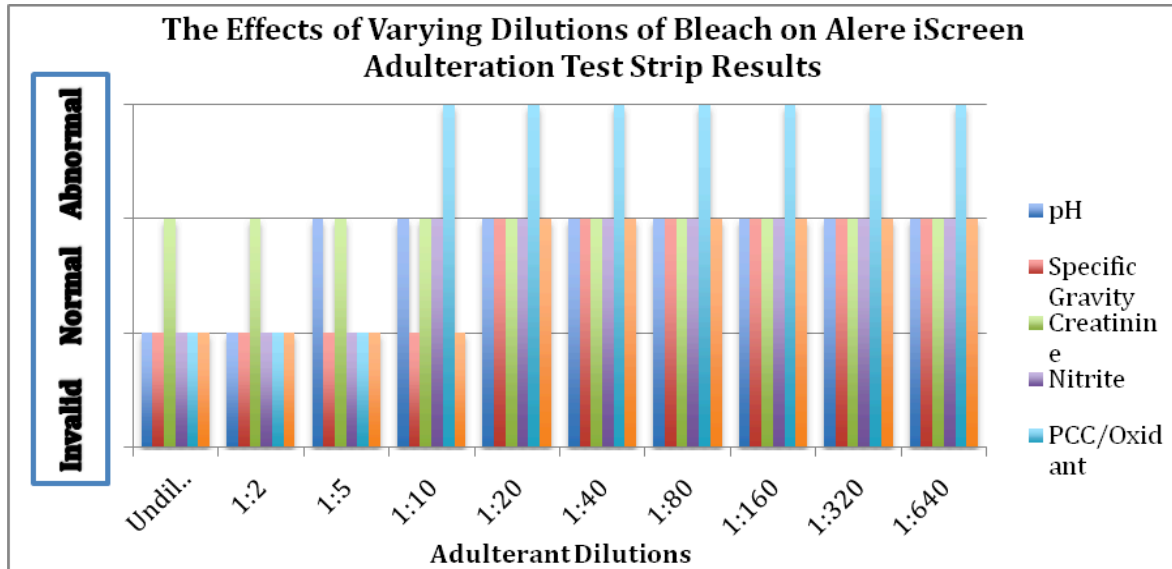
In the experimentation, the positive and negative urine controls were first run unadulterated to ensure the adulteration test strips were performing accurately. Another control of equal parts 0.85% saline and positive urine control was also run to ensure the saline would not cause abnormal results on the test strips that could interfere with the experimentation. After all controls gave negative results for adulteration the positive control was used to produce the samples for testing. The six adulterants being tested were run separately, but a similar procedure was used for each trial. The adulterant would first be tested undiluted by labeling a sterile urine cup with the name of the adulterant being tested and by adding 0.25mLs of positive control to

each cup. If the adulterant was a liquid, 0.25mLs of undiluted adulterant were then added to the respectively labeled sample cup. For the solid meat tenderizer, 2.5mg of the powder was weighed out and added to 0.25mL of positive control to create a solution equivalent to a concentration of 10mg/mL. This concentration was selected due to its description in the literature as effective at masking drug presence.⁶ The samples were gently swirled to create a homogenous solution. After mixing, the Alere iScreen© Urine Adulteration Test Strips were used to test for the presence of adulterants in each sample. Each sample was tested in duplicate and each trial's results for pH, specific gravity, nitrite, glutaraldehyde, creatinine, and pyridinium chlorochromate/oxidant presence were logged on a recording sheet. If positive for adulteration, the same procedure was then repeated by creating the appropriate dilutions for each adulterant and testing until either normal results or the decided upon dilution endpoint was reached. The appropriate endpoints and dilutions to be used were decided upon by evaluating the methods used by similar research sources.⁶

Data collected from the experimentation was semi-quantitative in nature and was measured by the test strips in the following units: the pH scale (2-10), specific gravity scale (1.000-1.030), positive/negative (glutaraldehyde and PCC/oxidant), and mg/dL (nitrite and creatinine). Normal and abnormal values for each test on the adulteration strip were given by the manufacturer's product insert, and this information was used to organize the data collected into three qualitative criteria: Normal, Abnormal, or Invalid. The use of qualitative data ruled out the need for statistical calculations to verify the validity of the hypothesis. Instead, compounded bar charts were created in Microsoft Excel to show comprehensive results for each adulterant tested and their relation to the hypothesis.

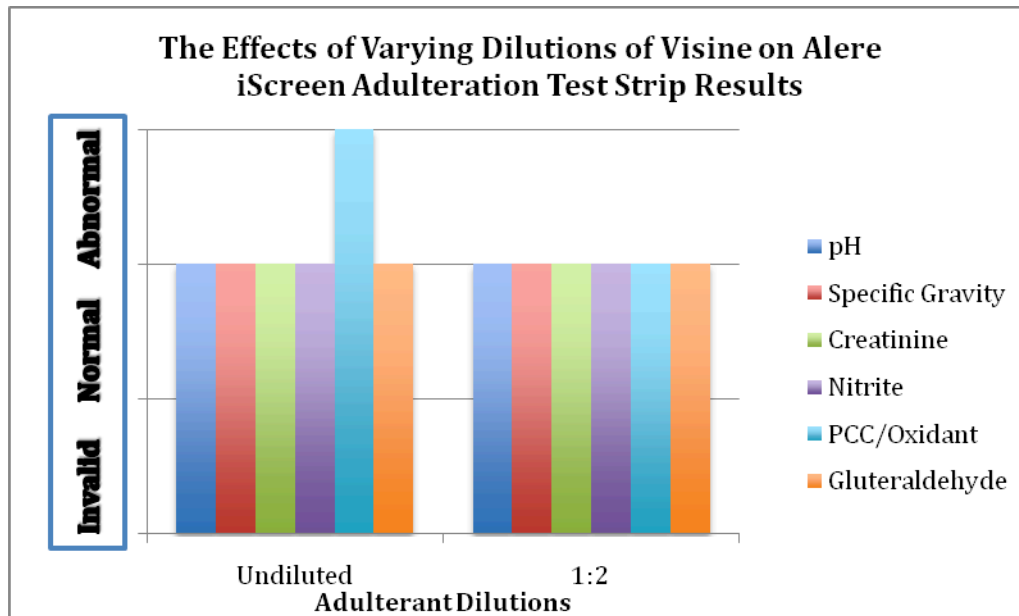
Results

BLEACH



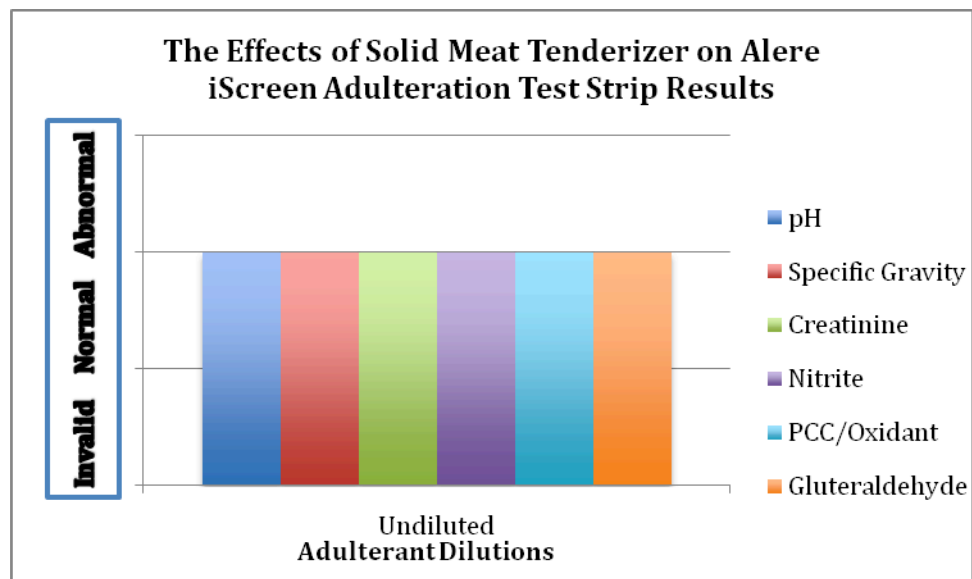
The addition of undiluted bleach to the positive urine control created invalid test results in all testing methods except for creatinine. This pattern was also seen when a 1:2 dilution of bleach was used. At a 1:5 dilution, the pH and creatinine gave normal results, but all other tests continued to be invalid due to the extremely basic solution. The 1:10 dilution gave invalid results for specific gravity and gluteraldehyde, normal results for pH, creatinine, and nitrite, and abnormal results for PCC/oxidant presence. The dilutions ranging from 1:20 to 1:640 gave abnormal test results for PCC/oxidant presence but all other tests were normal.

VISINE EYE DROPS



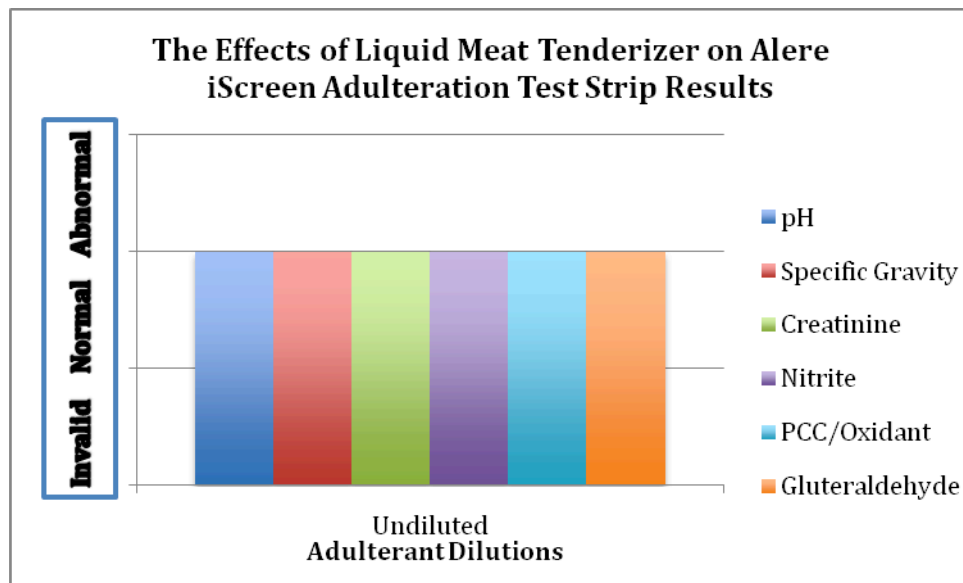
Testing undiluted Visine eye drops with the positive urine control resulted in normal values for all testing criteria except for PCC/oxidant presence. When a 1:2 dilution of the Visine was performed all results were normal.

SOLID MEAT TENDERIZER



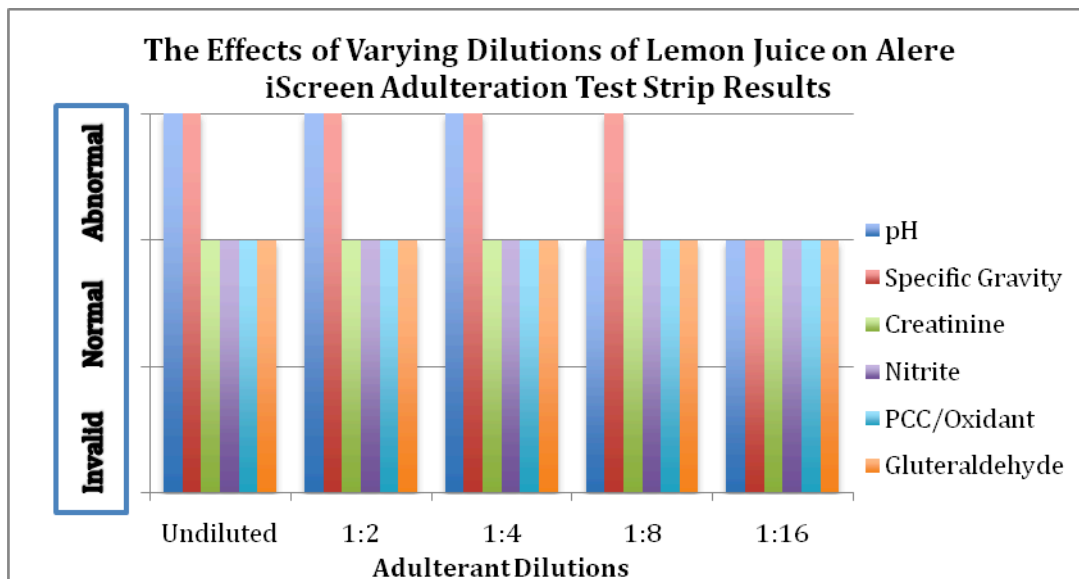
The adulteration strips could not detect the presence of undiluted powdered meat tenderizer in a concentration equivalent to 10mg/mL. All results were normal and no further testing was needed.

LIQUID MEAT TENDERIZER



Liquid meat tenderizer was added undiluted to the human urine control and all tests gave normal results. No further testing was necessary.

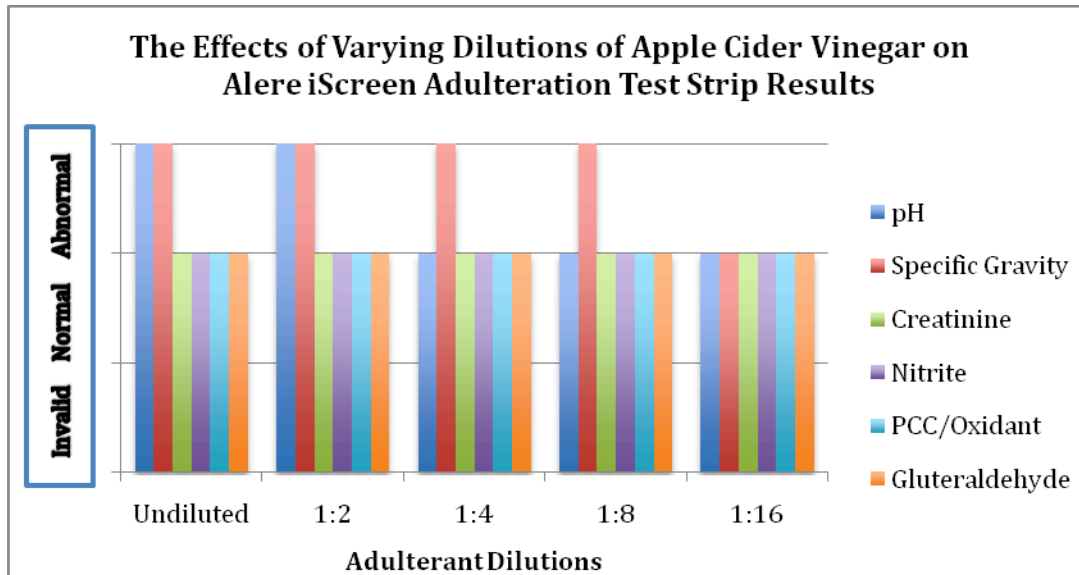
LEMON JUICE



Undiluted lemon juice added to the positive control gave abnormal results for pH and specific gravity. Dilutions of 1:2 and 1:4 also gave abnormal pH and specific gravity values. At a 1:8

dilution, the sample adulterated with lemon juice was only gave abnormal results for specific gravity. The 1:16 dilution gave all normal results.

APPLE CIDER VINEGAR



The undiluted vinegar specimen initially gave abnormal results for specific gravity and pH. The 1:2 dilution tested also gave these same results. When diluted to 1:4 and 1:8, the samples gave normal results for all criteria except for specific gravity. At a dilution of 1:16, all results were normal.

Discussion

The results of testing the Alere iScreen© Urine Adulteration Test Strips demonstrated that the commonly used household adulterants bleach, eye drops, lemon juice, and apple cider vinegar were capable of being detected by the test strips. Oftentimes, the same attributes that help these adulterants mask illicit drugs in urine samples are also what cause them to be detected by simple specimen validity tests. Bleach, or sodium hypochlorite, is a strong base that raises the pH of the specimen, causing interferences with drug binding in immunoassays.² While deemed effective at causing false immunoassay results, the basicity of the bleach caused invalid and

abnormal results on the iScreen© adulteration strips. The initial dilutions gave erroneous color readings on the strips that signified invalid test results and would be quickly identified as an adulterated sample. Even when diluted out to 1:640, the bleach still strongly affected the strips and gave abnormally high values on the pH scale. These results indicate that bleach is easily detected by the strips and would not be useful in a clinical setting where iScreen© Adulteration Test Strips are also used.

Lemon juice and apple cider vinegar act similarly to bleach in that they have strong effects on the pH of the samples. Lemon juice contains citric acid, while apple cider vinegar is a more dilute form of acetic acid. Both of these adulterants will lower the pH of a sample when they are added, affecting the binding reactions and solubility of drug immunoassay testing.² The effects of the acids on pH were detected by the iScreen© strips. Apple cider vinegar gave abnormal pH results to a dilution of 1:2 while lemon juice gave abnormal results to a dilution of 1:4. Specific gravity was also affected by lemon juice and vinegar, giving abnormal results to a dilution of 1:8 for both adulterants. Specific gravity evaluates the amount of dissolved substances present in a solution, and will increase as concentration and density increase.⁷ Since both lemon juice and apple cider vinegar typically have specific gravities greater than that of normal human urine, the addition of these substances to urine would become noticeable upon use of the iScreen© adulteration strips.

Visine eye drops are capable of causing false-negatives due to its inactive ingredients benzalkonium chloride and borate. These chemicals have been found to reduce the binding interactions that take place in immunoassays.² The undiluted eye drops gave only one abnormal test result, a positive reaction for PCC/Oxidant presence, which corrected when diluted 1:2. These results contradict the findings of previous research, where it has generally been concluded

that Visine eye drops are not detectable by point-of-care specimen validity tests.³ This unusual result could have been caused by an oxidizing agent present in the Visine eye drops that the iScreen© strips were sensitive enough to detect. Another possibility is that the abnormal reading could have been caused by human error due to color similarity between normal and low abnormal results on the iScreen© adulteration strips.

Meat tenderizer is a relatively new method of adulteration and has been found to create false negatives on immunoassay tests, specifically for metabolites of marijuana. False negatives results are caused by the active ingredient papain, a cysteine protease capable of cleaving peptide bonds of basic amino acids and hydrolyzing esters and amides.⁶ Adulteration with undiluted liquid and solid papain-based meat tenderizers gave normal results on the iScreen© test strips. The results of this research support the findings of similar studies done on papain-based adulterants, indicating that meat tenderizers are capable of avoiding detection by commercial specimen validity testing methods.⁸

Limitations

As in all research, definite limitations existed that affected the experimentation. A primary constraint was that manufactured urine controls were used instead of human drug-positive urine samples. The volume of control used to create testing samples was also very small and not comparable to actual sample volumes seen in a laboratory setting. The use of dilutions, although useful for determining sensitivity of the adulteration test strips, would also not be typically seen in a real-life adulteration scenario. Another drawback to the use of dilutions is that due to limitations in budget and time it was not possible to correlate by immunoassay if the adulterant dilutions used would mask the presence of illicit drugs in the sample. Lastly, human

error is always an element of research and could have affected the results due to errors in pipetting and visual reading of the test strip reactions.

Future Research

This research covers only one aspect of urine adulteration testing and could easily be expanded upon in the future. Use of clinical human urine samples instead of stabilized controls is one way to expand the scope of this research. It would also be possible to test commercially produced adulterants used to pass drug tests instead of the common household adulterants utilized in this research. Correlation of results of adulterated urine samples on drug-of-abuse immunoassays and specimen validity testing is another available option. Lastly, a wide variety of adulteration test strips are available in the marketplace. Future research could compare the effectiveness of other such methods to the iScreen© Urine Adulteration Test Strips or evaluate them on an individual basis.

Acknowledgements

The author wishes to acknowledge Mrs. Ann McConnell, Dr. Ann Mannie, Ms. Cheryl Binkley and the East Carolina University Department of Biostatistics for their mentorship and assistance in the research process. Thanks are also given to Ahmad Alqarni, Amanda Schaeffer, Jeffrey Smith, and Latisha Waddell for their previous work that provided the foundation for this research. The Department of Clinical Laboratory Science and Honors College of East Carolina University supported this research.

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